

REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and following remarks.

Claims 1 and 7 have been amended to incorporate the subject matter of claim 4. The amendments to claim 4 are supported in the specification at page 9, line 18. Claim 6 has been amended so as to be dependent upon claim 1. Claim 9 has been amended so as to be dependent upon claim 7. The amendments to claims 11 and 12 are supported by the amendments to claim 4. Claim 16 has been amended for clarification purposes and is supported by page 13, line 4 to page 14, line 3 of the specification.

New claims 18-20 are added and are supported by claims 4 and 15.

Turning to the Official Action, claims 1-3, 5, 6-10 and 13-17 are rejected under 35 USC 102 as anticipated by Lerner et al.

This ground of rejection is deemed to be overcome by incorporation of the subject matter of non-rejected claim 4 into claims 1 and 7 and by amendment of claims 6 and 9 to be dependent upon claims 1 and 7, respectively.

Claims 4, 11 and 12 are rejected under 35 USC 103 as unpatentable over Lerner et al. This ground of rejection is respectfully traversed.

The present invention is characterized by the control of release of the medicament in the intestine, particularly so as to release the medicament more in the large intestine by utilizing the property of chitosan as is explained in more detail below.

(A) The Examiner says in item 8 of the Office Action that "as discussed above the '332 reference discloses a solid formulation comprising a core with a successive coating of a water-insoluble polymer with chitosan dispersed therein". However, there is no specific description in the reference of using chitosan as the water-insoluble hydrophilic particulate matter. Instead, only calcium pectinate (Ex. 1-5, and 9), crospovidone (Ex. 7), crosslinked byco (Ex. 6), and microcrystalline cellulose (Ex. 8) are exemplified as the hydrophilic particulate.

That is, Lerner et al. disclose a drug delivery formulation for localized drug release in the gastrointestinal tract which comprises (a) a core comprising a drug and core material, and (b) a

coating surrounding said core with a water-insoluble hydrophilic particulate matter embedded in a water-insoluble carrier, and optionally (c) an enteric coating. The reference discloses many materials as the water-insoluble hydrophilic particulate material for the coating components in col. 9, lines 38-62 among which chitosan is included (col. 9, line 48).

It should be noted, however, that chitosan is not water-insoluble but is soluble in acidic water. (Please see the attached copy of "Carbohydrate Polymers 36 (1998), pp. 121-127", particularly page 121, right column, lines 21-25).

Thus, although Lerner et al. '332 reference discloses many particulate materials (about 30 kinds of materials; cf. Lerner et al. '332, col. 9, lines 7-17 & 46-62), only four kinds of the particulate materials as mentioned above are used in the examples, which are different from chitosan in solubility in water. Even if chitosan is accidentally mentioned as one of the water-insoluble hydrophilic particle materials in Lerner et al., it is distinguished from the specifically disclosed water-insoluble hydrophilic particulate materials in view of the solubility in water as mentioned above.

Accordingly, it is respectfully submitted that a person skilled in the art would have never expected or predicted the properties of a solid preparation having dispersed chitosan in a water-insoluble polymer from the teachings of the reference.

(B) On the other hand, according to the present invention, by utilizing the specific property of chitosan, there is obtained the desired solid preparation having the well controlled release of the medicament in the intestine, particularly in the large intestine.

That is, the present invention provides a colonic delivery solid preparation comprising (i) a core comprising a medicament-containing solid material, (ii) an inner coating layer of a water-insoluble polymer wherein a chitosan powder is dispersed in the ratio of 1:10 to 10:1 by weight and (iii) an outer enteric coating layer of an enteric polymer (as claimed in the product claims 1-5 and 14-15 as well as the process claim 6), and a sustained release solid preparation comprising (i) a core comprising a medicament-containing solid material and (ii) a single coating layer of a water-insoluble polymer wherein a chitosan powder is dispersed in the ratio of 1:10 to 10:1 by weight (as claimed in the product claims 7-8 and 16 and a process claim 9).

(B)-1. According to the colonic delivery solid preparation of claim 1, the preparation is not dissolved within the stomach owing to the enteric coating and it passes through the small intestine, where the preparation is not disintegrated due to the property of chitosan contained in the inner coating layer with chitosan powder-dispersed water-insoluble polymer. Since chitosan is soluble in an acidic aqueous medium but insoluble in neutral or alkaline aqueous medium, even after the enteric coating is removed (decomposed) in the upper part of the small intestine, the medicament-containing core is still covered by the inner coating layer of the chitosan-dispersed water-insoluble polymer wherein chitosan is not dissolved or swollen at all with the intestine juice (having an almost neutral pH value, cf. please see the attached copy of "USP XXII, page 1789, Intestinal Fluid, Simulated, TS"). And when the solid preparation reaches to the region of the large intestine (colon), chitosan is rapidly decomposed by *E. coli* and a small amount of water having a pH about 4.0 contained in the large intestine. Thereby many pores are formed in the inner coating layer. As a result, the active medicament is rapidly released through pores in the inner coating layer.

The excellent effects of the colonic delivery solid preparation have been experimentally confirmed as shown in Fig. 9 of the present application, which shows the relation between the dissolution percentage of the medicament and the time of the colonic delivery pellet preparation of Example 7.

Thus, according to the colonic delivery solid preparation having an enteric coating of the present invention, by utilizing the property of chitosan, it has well controlled releasing characteristic and can release the medicament more in the large intestine. When the kind and amount of the water-insoluble polymer and chitosan powder are varied, the starting point and the speed of the release of medicament can be controlled. Accordingly, the delivery of medicament can specifically be controlled with respect to the region to be released as well as the time for release, and thereby the release of medicament can be controlled in the wide range of from the small intestine to the large intestine and further the rate of release is also well controlled.

(B)-2. Furthermore, when the solid preparation in the form of a sustained release preparation according to claim 7 is orally administered, the chitosan powder dispersed within the

water-insoluble polymer coating layer is partially dissolved by an acidic stomach juice in the stomach to give some pores in the coating layer, through which the medicament contained in the core is partly released out in the stomach. Further, during passing through the small intestine, the medicament is continuously released through the pores while chitosan is not dissolved at all. After the solid preparation reaches to the large intestine tract, chitosan powder is specifically decomposed by *E. coli* and a small amount of water having a pH about 4.0 contained in the large intestine. Thereby many pores are formed in the inner coating layer. As a result, the active medicament is rapidly released through pores in the inner coating layer. The excellent effects of the sustained release solid preparation have been experimentally confirmed as shown in Fig. 7 of the present application, which shows the relation between the dissolution percentage of the medicament and the time of the sustained release pellet preparation of Example 6.

Thus, in the sustained release solid preparation, the release of medicament can also be controlled in the wide range through the gastrointestinal tracts such as the stomach, the small intestine and the large intestine in terms of the release rate and amount of the medicament.

(C) It should also be noted that Lerner et al. teaches "For colonic administration, the formulation is designed so as to prevent release of the drug in the stomach and small intestine environments and to be unaffected by the stomach and small intestine enzymes. When targeting the colon, the preferred embodiment has the advantage of the guaranteed release of the drug load since the major portion of the coating system (70% calcium pectinate) and the core (calcium pectinate and pectin) are enzymatically degradable in the colon while being non-degradable by enzymes of the stomach and small intestine".

Thus, Lerner et al. teaches away from the invention in preventing the release of the medicament in the stomach by coating the core with a water-insoluble particulate material embedded in a water-insoluble carrier, even though no enteric coating is applied. This is clearly distinguished from the present invention, wherein the chitosan powder dispersed within the water-insoluble polymer coating layer is partially dissolved by an acidic stomach juice in the stomach to give some pores in the coating layer, through which the medicament contained in the core is partly released out in the stomach.

As is clear from the above explanation, the characteristic features and excellent effects of the present invention are not taught or suggested by Lerner et al.

In view of the foregoing, it is respectfully submitted that the claimed invention is patentably distinct and not obvious from the teachings of the cited reference. Accordingly, reconsideration and allowance is solicited.

Respectfully submitted,

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Solubility parameter of chitin and chitosan¹

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Abstract

The solubility parameter of chitin and chitosan was determined by group contribution methods (GCM) and the values were compared with the values determined from maximum intrinsic viscosity, surface tension, the Flory–Huggins interaction parameter and dielectric constant values. The values, thus, obtained were confirmed by values obtained from GCM. The solubility parameters of chitosan determined by these methods are more or less equal and the average is approximately $41 \text{ J}^{1/2}/\text{cm}^{3/2}$. A method for estimating the overall solubility parameter of chitosan having any degree of deacetylation is proposed. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: Chitosan; Solubility parameter; Chitin

1. Introduction

The solubility parameter (δ) is an important property of polymers which is defined as the square root of cohesive energy density. In low molecular weight compounds it is an indication of the heats of vaporization. There are several methods to estimate δ by group contribution methods, proposed by various investigators (Hoflyzer and Van Krevelen, 1976; Van Krevelen, 1965; Hoy, 1970; Small, 1953; Hansen, 1967; Hildebrand, 1916). Owing to some specific properties, deviations were found in δ values determined by group contribution method when compared with experimental or physical methods. The main advantage of group contribution methods is that it is easy to estimate individual contributions such as dispersive (δ_d), polar (δ_p), and hydrogen bonding (δ_h) of polymers/low molecular weight compounds, through which the overall solubility parameter (δ_t) can be estimated by using Eq. (1)

$$\delta_t = \sqrt{\delta_d^2 + \delta_p^2 + \delta_h^2} \quad (1)$$

In many instances, the physical properties of polymers are found to correlate strongly with interconnections between the atoms of a molecule. The values of heats of vaporization, molar refraction, molar volume, refractive index, boiling point, specific gravity and viscoelasticity are mainly governed by the molecular topology of that particular compound (Rouvray and Pandey, 1986; Bonchev, 1983; Kier

and Hall, 1976). Hence, an attempt was made to determine the δ_t of polymer δ_{pt} by the maximum intrinsic viscosity number (MIV) (Garden, 1965), surface tension measurement (Thomos, 1975), refractive index (Mandel et al., 1982), dielectric constant (Darbye et al., 1967), and Flory–Huggins interaction parameter (Shultz and Glory, 1955).

Chitosan, a transformed oligosaccharide, is obtained by deacetylation of chitin, the latter being a bio-polymer obtained from crab and shrimp shells. Chitin is a pure polymerized form of *N*-acetyl glucosamine, as shown by the structure in Fig. 1. It has drawn more attention than other bio-polymers because of its ability to form specific complexes with number of ions or dyes as well as specific complexes with organic molecules (Muzzarelli, 1973a; Muzzarelli, 1973b; Roberts, 1992). A fraction of the repeating units in the chitosan backbone contains $-\text{NH}_2$ pendant groups while the rest contains acetamide group ($-\text{NHCO}-$) in its place. The degree of deacetylation can be controlled by time, temperature and concentration of alkaline treatment of chitin (Struszyk, 1987). Chitin and chitosan are practically insoluble in many of the organic or inorganic compounds, but soluble in salt organic mixtures of LiCl – N,N -DMAc (Striegel and Timpa, 1995), and dilute acids (water–acid mixtures). It is worth mentioning that no information is available in literature regarding δ_t of chitosan. Hence, the objective of this study is to determine δ_t of chitosan by using well known and simple physical methods. Thus, the values of surface tension, and dielectric constant, of the polymer were determined by instrumental as well as

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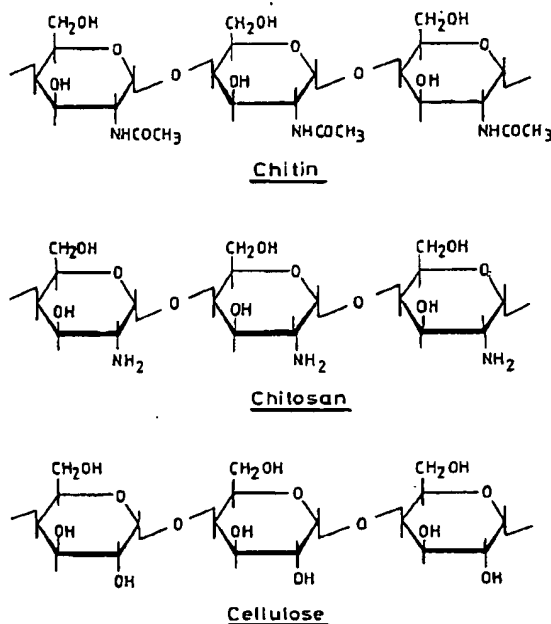


Fig. 1. Chemical structures of polysaccharides.

group contribution methods. Since it is also well known that no pure solvent can dissolve chitin or chitosan, polymer solutions were prepared by using mixtures of solvents. In the present context, 'solvent' refers to the solvent mixture of hydrochloric acid (HCl) and water, with varying compositions, and the value of δ_{p} is expressed in $\text{J}^{1/2}/\text{cm}^{3/2}$.

2. Experimental

2.1. Materials

Chitosan was purchased locally in India. Its viscosity average molecular weight M_v (Lee, 1974), was found to be $\approx 500\,000$ and the degree of deacetylation determined as 64% by the IR method (Sannon et al., 1978). Acetic acid

was purchased from Merck (India) limited. Double distilled water was used throughout the experimental work.

2.2. Purification of polymer

Chitosan was purified by dissolving in 2% aqueous acetic acid solution and precipitating in 10 wt% NaOH solution. The precipitate was first washed with distilled water to neutrality and then rinsed with acetone. It was then vacuum dried at 70°C for about 8 h. Its degree of deacetylation, determined by IR method (Sannon et al., 1978), was found to be 64%.

2.3. Membrane preparation

A known amount of purified chitosan was dissolved in 100 ml of 2 wt% aqueous acetic acid solution by continuous stirring to get a clear homogeneous polymer solution. The solution was cast on a clean glass plate to a desired thickness and the solvent was allowed to evaporate initially at room temperature and then the residual solvent was removed by vacuum drying. The membrane was peeled off from the glass plate and vacuum dried at ambient temperature.

2.4. Methods of estimation of δ by group contribution

Extensive work has been published on δ since Small (1953) put forward his now famous method based on the contributions owing to individual groups, atoms and bonds constituting the molecules. Similar methodologies were advanced by other researchers. Among those, of special significance are Van Krevelen (1965), Hoftyzer and Van Krevelen (1976), and Hoy (1970). Another method of estimation of δ is by the cohesive energy densities of groups. However, this method is not so suitable for strong hydrogen bonding materials as in the present case and, hence, it is not discussed here. All the above stated methods are applicable to both low and high molecular weight compounds, but some corrections need to be applied.

Table 1
Values of molar volumes (at room temperature, in cm^3/mol) proposed by Fedors

Group	V_i		V_i	
	Chitin	Chitosan	Chitin	Chitosan
-O-	3.8	3.8	7.6	7.6
-OH (primary)	10	10	10	10
-OH ^a	13	13	13	13
-CH ₂	16.1	16.1	16.1	16.1
-CH	-1	-5	-1	-5
-NH ₂	—	19.2	—	19.2
-NHCO	9.5	—	9.5	—
-CH ₃	33.5	—	33.5	—
Ring structure	16.0	16.0	16.0	16.0

^aSecondary or adjacent carbon atom.

ΣV_i for pure chitin = 100.7.

ΣV_i for pure chitosan = 76.9.

Table 2

Values of molar attraction constant of various groups proposed by Van Krevelen and Hoy

Group	Van Krevelen				Hoy			
	F_i		F_i		F_i		F_i	
-O-	256	256	512	512	235.5	235.5	470.6	470.6
-OH	754	754	1508	1508	462.0	462.0	924.0	924.0
-CH ₂	280	280	280	280	269.0	269.0	269.0	269.0
-CH	140	140	700	700	176	176	880	880
-CH ₃	420	—	420	—	303.4	—	303.4	—
-CONH	1228	—	1228	—	906.4	—	906.4	—
-NH ₂	—	683	—	683	—	595	—	595

2.5. The molar volumes of chitin and chitosan

Molar volume (V) is the product of specific volume (ν) and molecular weight (M) of the repeating unit as given below.

$$V = (M)(\nu) = \frac{M}{\rho} \quad (2)$$

where ρ is the density.

Molar volume is the basic value needed for estimating the δ values of chitin and chitosan by applying various methods. V value contributions of specific groups in their molecular structures are given by Fedors (1974). Based on the structures of chitin and completely deacetylated chitosan, one can estimate the V value again by Fedor's group contribution method. Chitin consists of -CH, -OH, -CH₂, -NHCO-, -CH₃, whereas chitosan in completely deacetylated state (CDA) contains only -NH, and no -NHCO- groups. These are schematically shown in Fig. 1. The estimated values are compared with published values of cellulose and ethyl cellulose which are similar in structure. Table 1 gives V values of various groups of chitin/chitosan proposed by Fedors. Total V value, i.e. V_1 , of chitin and chitosan was estimated to be 100.7 and 76.9 in cm³/mol.

2.6. Estimation of δ from molar attraction constants: Hoy and Van Krevelen's method

The contribution values to the molar attraction constant

(F), or individual (F_i) and total (F_1) for groups contained in chitin and chitosan are presented from the values published by Hoy and Van Krevelen in Table 2. The total molar attraction constant (ΣF_i) of a group in a molecules is obtained from

$$F_1 = (\Sigma F_i)(N_i) \quad (3)$$

where N_i is the number of groups i present in the molecule. F_1 for pure chitin is found to be 4648 by Van Krevelen's and 3753.4 by Hoy's contribution methods. Similarly, the values for pure chitosan are obtained as 3683 using Van Krevelen and 3138.6 by Hoy values. The solubility parameter is defined by

$$\delta_1 = \frac{F_1}{V} \quad (4)$$

and from Table 1, δ_{pt} of CDA chitosan equals to 47.89 by Van Krevelen's method and it is 40.814 by Hoy's method. Similarly, δ_{pt} for pure chitin is 46.157 by Van Krevelen's method and is 36.57 by Hoy's method.

The methods of Hoy and Van Krevelen can give only the overall solubility parameter value. To determine the individual δ_p , δ_d and δ_h values, the methods of Hoftyzer and Van Krevelen (1970), and Hoy's system (Hoy, 1985) are particularly useful. The dispersion component of the molar attraction constant (F_{pi}) and contribution of the hydrogen bonding forces to the cohesive energy (E_{hi}) and E_{bi} (total) for the groups from the Hoftyzer–Van Krevelen method are given in Table 3. The calculated values of δ_p , δ_d , and δ_h along with the δ , are given in Table 4 and compared with

Table 3

Values of F_d , F_p (in J^{1/2}/cm^{3/2}/mol) and E_{hi} (in J/mol) proposed by Hoftyzer–Van Krevelen

Group	F_d		F_d		F_{pi}		F_{pi}		E_{hi}		E_{bi}	
	Chitin	Chitosan	Chitin	Chitosan	Chitin	Chitosan	Chitin	Chitosan	Chitin	Chitosan	Chitin	Chitosan
-O	100	100	200	200	400	400	800	800	3000	3000	6000	6000
-OH	210	210	420	420	500	500	1000	1000	20000	20000	40000	40000
-CH ₂	270	270	270	270	0	0	0	0	0	0	0	0
-CH	80	80	400	400	0	0	0	0	0	0	0	0
-NH ₂	—	280	—	280	—	310	—	310	—	8400	—	8400
-NH	160	—	160	—	210	—	210	—	3100	—	3100	—
-C=O	290	—	290	—	770	—	770	—	2000	—	2000	—
CH ₃	420	—	420	—	0	—	0	—	0	—	0	—
Ring structure	190	190	190	190	0	—	0	—	0	—	0	—

Table 4

Values δ_d , δ_p , and δ_h ($J^{1/2}/cm^{3/2}$) obtained from Hoflyzer–Van Krevelen method and Hoy's system

Polymer	δ_d		δ		δ	
	Hoflyzer–Van Krevelen method	Hoy's system	Hoflyzer–Van Krevelen method	Hoy's system	Hoflyzer–Van Krevelen method	Hoy's system
Chitin	23.887	23.323	17.985	23.28	22.257	26.49
Chitosan	22.807	29.22	17.134	26.492	26.597	24.156

the values obtained by the Hoy's system. These are estimated according to the following equations

$$\delta_d = \frac{\sum F_{di}}{V} \quad (5)$$

$$\delta_p = \frac{\sqrt{(\sum F_{pi})^2}}{V} \text{ and} \quad (6)$$

$$\delta_h = \sqrt{\frac{\sum F_{hi}}{V}} \quad (7)$$

δ_i is given by Eq. (1).

2.7. Estimation of δ from molar attraction constants by Hoy's system

As mentioned above, this is an alternative method to determine individual values of δ_d , δ_p and δ_h which is somewhat different from that of Hoflyzer–Van Krevelen. Table 5 lists constants for three additive molar functions: molar attraction constant (F_i), polar components (P_p) and Cyderson correction ($\Delta_i^{(p)}$) for polymer. These values are to be used in auxiliary equations and the expression for δ_i . The values of various groups are given in Table 5. The molecular aggregation number for the polymer is given by

$$\alpha^{(p)} = \frac{777 \sum \Delta_i^{(p)}}{V} \quad (8)$$

where 777 is a constant and V is molar volume. Substituting

the values from Table 5 we obtain $\alpha^{(p)}$ as 2.188 and 2.1335 for chitosan and chitin, respectively.

The number of repeating units per effective chain segment of the polymer is given by

$$n = \frac{0.5}{\Delta_T^{(p)}} \quad (9)$$

and is obtained as 2.3095 and 1.808 for chitosan and chitin, respectively. The contributions of δ_d , δ_p and δ_h are obtained from

$$\delta_i = \frac{F_i + \left(\frac{B}{n}\right)}{V} \quad (10)$$

$$\delta_p = \delta_i \left[\left(\frac{1}{\alpha^{(p)}} \right) \left(\frac{F_p}{F_i + \frac{B}{n}} \right) \right]^{\frac{1}{2}} \quad (11)$$

$$\delta_h = \delta_i \left(\frac{\alpha^{(p)} - 1}{\alpha^{(p)}} \right)^{\frac{1}{2}} \quad (12)$$

$$\delta_d = (\delta_i^2 + \delta_p^2 + \delta_h^2)^{\frac{1}{2}} \quad (13)$$

Based on the above average δ_p values of pure chitin and chitosan, one can estimate the overall solubility of chitosan having any other degree of deacetylation. For example, the δ_{pi} of chitosan having $X\%$ of degree of acetylation can be

Table 5

Values of F_i , F_p and $\Delta_i^{(p)}$ proposed by Hoy's system

Group	F_i		F_h		F_{pi}		F_{pi}		$\Delta_i^{(p)}$		Δ_i corrected for both	$\Delta_i^{(p)}$	
	Chitin	Chitosan	Chitin	Chitosan	Chitin	Chitosan	Chitin	Chitosan	Chitin	Chitosan		Chitin	Chitosan
-O-	235	235	470	470	216	216	432	432	0.018	0.018	(x)2/3	0.024	0.024
-OH (primary)	675	675	675	675	675	675	675	675	0.049	0.049	—	0.049	0.049
-OH (secondary)	591	591	591	591	591	591	591	591	0.049	0.049	—	0.049	0.049
-CH ₃	269	269	269	269	0	0	0	0	0.020	0.020	—	0.020	0.020
-CH	176	176	880	880	0	0	0	0	0.013	0.013	(x)2/3	0.043	0.043
-CONH	1131	—	1131	—	895	—	895	—	0.073	—	—	0.073	—
-CH ₂	303.5	—	303.5	—	0	—	0	—	0.022	—	—	0.022	—
-NH ₂	—	464	—	464	—	464	—	464	—	0.035	—	—	0.035
Ring structure	-48	-48	-48	-48	61	61	61	61	-0.0035	-0.0035	—	-0.0035	-0.0035

For bi, tri, and tetra valent group in saturated ring the $\Delta_i^{(p)}$ values should be multiplied (\times) by a value 2/3.

*The values are given in (J/cm^3)^{1/2}/mol.

Table 6
Overall solubility parameter (δ_i) obtained by various methods in $J^{1/2}/cm^{3/2}$

Polymer	Hoy	Van Krevelen	Hoflyzer–Van Krevelen	Hoy's system	Average δ_i
Chitin	37.27	46.16	37.28	43.9	41.15
Chitosan	40.81	47.89	39.05	44.49	43.06

obtained as

$$= [\delta_i \text{ of 100\% chitosan} \left(\frac{X}{100} \right)] \\ + [\delta_i \text{ of chitin} (1 - \left(\frac{X}{100} \right))] \text{ (for chitosan)} \quad (14)$$

The ratio ($X/100$) represents the molar consent of 100% chitosan present as a fraction in the chitosan of interest. Likewise, $(1 - X/100)$ represents the fraction of chitin units present in the molecule.

It appears from Table 6 that Hoy's and Hoflyzer–Van Krevelen methods give similar δ_i values in this particular case, whereas the values by Van Krevelen method and Hoy's system are closer. These predictions will be compared with the experimentally obtained values for further evaluation of the various group contribution methods.

2.8. Maximum intrinsic viscosity value (MIV)

Generally, polymers dissolve in various solvents and their δ_i value can be estimated by knowing the maximum swelling index value. Compatibility of any polymer with the solvent(s) is estimated by its intrinsic viscosity $[\eta]$ value which is equal to

$$[\eta]_{c \rightarrow 0} = \lim_{c \rightarrow 0} (\eta_{sp}/c) = \lim \eta_{inh} \quad (15)$$

where η_{sp}/c is specific viscosity at concentration 'c' and η_{inh} is inherent viscosity. In either case, the value is obtained as the zero concentration intercept of the extrapolated curve of η_{sp}/c or η_{inh} versus concentration. Viscosity is determined by using Ubbelohde viscometer. The estimation of MIV is complicated in the case of chitosan, since the polymer does not dissolve in any pure solvent, but dissolves in mixture of solvents like aqueous acid solutions at room temperatures or solvent and salt mixtures like DMAc–LiCl at higher temperatures. In the former case, the solubility parameter of the mixture or solvents can be determined by using the formula

$$\delta_{sol} = \nu_1 \delta_1 + \nu_2 \delta_2 \quad (16)$$

where ν and δ are volume fraction and solubility parameters of component 1 (water) and 2 (HCl) in the mixture of

solvents. In the present study, the concentration of HCl in water–HCl solution is varied from 2–50 vol%.

2.9. Surface tension measurements

The surface tension (γ) is a manifestation of intermolecular forces. It is related to other properties derived from intermolecular forces, such as internal pressure, compressibility and cohesion energy density. γ is an important tool for measuring the interaction capacity of the solvent(s) with the polymer. This value is minimum when the polymer and solvent are highly compatible. Known concentrations of polymer solutions were prepared by dissolving in different water–HCl solvent solutions, whose δ_{sol} can be calculated from Eq. (16). γ values of the resulting polymer solutions were determined by using torsion balance (White Elec. Inst. Co.). Experiments were repeated three times for each polymer solution, and the average value was taken into consideration.

2.10. Calculation of surface tension from an additive function, the parachor

The molar parachor is a useful means of estimating surface tensions. It is expressed as

$$\sum P_s = \gamma^{1/4} V \quad (17)$$

where P_s is the parachor value which was introduced by Suggen (1924), who gave a list of atomic constants from which the values of atoms that constitute chitin/chitosan is shown in Table 7, and V is the molar volume which is equal to 100.7 for chitin and 76.9 for chitosan. After calculating the γ of the polymer, its solubility parameter could be calculated by using Eq. (18)

$$\delta = \gamma^{3/4} \quad (18)$$

2.11. Correlation between dielectric constant and δ

Darbye et al. (1967) suggested a correlation between δ

Table 7
Prachor surface tension values proposed by Sugden

Group	CH ₂	C	O	H	N	Six-membered ring
P_{si}	39.0	4.8	20	17.1	12.5	6.1
P_{st}	39	33.6	100	188.1	12.5	6.1

Table 8

Molar polarization values proposed by Vogel

Group	-CONH	O	-CH ₃	-CH ₂	OH primary	OH other	CH
P_v	125	30	17.66	20.64	30	100	23.5
P_n	123	60	17.66	20.64	30	100	117.5

and the electric forces resulting from polarizability and polar moment that determine the cohesive energy, with which δ can be directly determined using Eq. (19).

$$\delta \approx 7.0\varepsilon \quad (19)$$

where ε is the dielectric constant of polymer. The ε value was determined experimentally by using HP-4192A LF impedance analyzer by applying biased voltage equal to one. The frequency was varied from 1 to 450 KHz. ε can also be calculated from molar polarization (P_v). The P_v of a dielectric can be defined as follows.

$$\sum P_v = \varepsilon^{1/2} M$$

where M is molar mass per structural unit. The value of P_v can be calculated by using a group contribution method proposed by Vogel. Some of individual P_v values of different atoms and molecules are cited in the text of Van Krevelen (1990), and are given in Table 8.

2.12. Flory-Huggins interaction parameter (χ)

Sorption equilibrium between a swollen macromolecular coil and a component solvent considerably affects the thermodynamic, transport, rheological and optical properties of the polymer. A thermodynamic interpretation of data on preferential and total sorption is usually based on Flory-Huggins interaction parameter (χ). The δ of polymer can be estimated by inserting the value of χ in the following equation Shultz and Glory (1955);

$$\chi = (\delta_1 - \delta_{pl})^2 \left(\frac{V_1}{RT} \right) \quad (20)$$

where V_1 is molar volume of polymer, T is temperature in

Kelvin at which the experiment is conducted, R is the universal gas constant, δ_1 and δ_{pl} are solubility parameters of the solvent and polymer, respectively. δ_{pl} can be estimated by known value of χ which can be determined by the formula written as (Shultz and Glory, 1955);

$$\chi = - \frac{[\ln(1 - \nu_p) + \nu_p]}{\nu_p^2} \quad (21)$$

where ν_p is the volume fraction of the polymer.

3. Results and discussions

Fig. 2 is a pictorial representation of $[\eta]$ versus δ_{sol} (solubility parameter of mixture of solvents). The maximum value of $[\eta]$, where the compatibility of solvent with polymer is maximum, is almost equal to the δ_1 of polymer. For the present study, the maximum value for $[\eta]$ is obtained at 30 vol% of HCl-water solution and, hence, the δ_{pl} is equal to 41.48.

The average γ value of the polymer dissolved in the solution of solvents having different δ_{sol} is shown in Fig. 3. Initially, the γ value constantly decreases with increase in δ_{sol} and then it increases steeply. Two separate lines are drawn joining the points where γ decreases and then from where it starts to increase. The corresponding δ_{sol} value on the X-axis where the two lines coincide is considered as δ_{pl} and is equal to 39.8. The calculated γ value of the polymer was originally derived from parachor values by group contribution method. Here, it is found to be 144.77. The solubility parameter was determined by inserting γ in Eq.

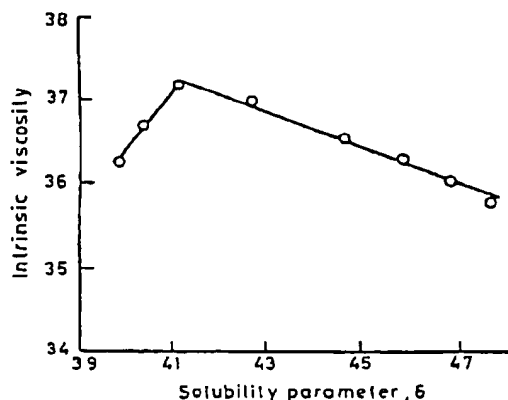
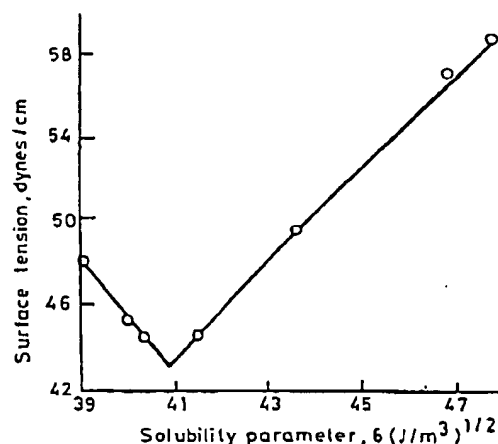
Fig. 2. Relationship between intrinsic viscosity and δ .Fig. 3. Relationship between surface tension and δ .

Table 9
Overall solubility parameter of chitosan

Method	MIV	γ	ϵ	χ	
				Water	Hydrazine
δ_{pi}	41.48	39.8	37.02	43.45	41.73

(18), then δ_p of polymer was calculated and is found to be equal to 42.38.

The experimentally determined ϵ values are similar in the frequency range of 10–450 KHz, and the average value was 5.29. Inserting this ϵ value in Eq. (19), the δ of polymer was found to be 37.02. From Table 8, P_v of chitosan is calculated which is equal to 5.37 and corresponding δ_{pi} is 37.65.

In the present case, the ν_p values of chitosan with water, and hydrazine are estimated. The values of χ were found to be 0.5917 and 0.951 for water and hydrazine, respectively. Inserting the value in Eq. (20) one can get the δ_{pi} of chitosan as 43.45 (from water) and 41.73 (from hydrazine).

4. Conclusions

In this first systematic attempt, the overall solubility parameter values of chitosan and chitin are estimated by Hoy, Van Krevelen, Hoftyzer–Van Krevelen, and Hoy systems. Results are tabulated in Table 6. The first two methods can yield δ_{pi} values only, whereas the latter two can also provide individual contributions of δ_d , δ_p and δ_h to the δ_{pi} values. The overall solubility parameter of chitosan polymer was also determined by maximum intrinsic viscosity, surface tension, dielectric constant, and Flory–Huggins interaction parameter values and the values are presented in Table 9. The average δ_i of 64% deacetylated chitosan was found to be 40.7. This value is in excellent agreement with the value of 42.366 obtained by applying group contribution method. Based on the δ_{pi} values of pure chitin and CDA chitosan, one can estimate δ_i for chitosan/chitin having any degree of deacetylation from Eq. (14).

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(see in the section, *Volumetric Solutions*) add water to make 250 mL. Add to the resulting solution an equal volume of sodium acetate solution freshly prepared by dissolving 13.66 g of anhydrous sodium acetate in water to make 500 mL and adjusting with 0.5 *N* acetic acid to a pH of 7. Store in a refrigerator, and use within 2 weeks.

Intestinal Fluid, Simulated, TS—Dissolve 6.8 g of monobasic potassium phosphate in 250 mL of water, mix, and add 190 mL of 0.2 *N* sodium hydroxide and 400 mL of water. Add 10.0 g of pancreatin, mix, and adjust the resulting solution with 0.2 *N* sodium hydroxide to a pH of 7.5 ± 0.1 . Dilute with water to 1000 mL.

Iodine TS—Use 0.1 *N* Iodine (see in the section, *Volumetric Solutions*).

Iodine Monochloride TS—Dissolve 10 g of potassium iodide and 6.44 g of potassium iodate in 75 mL of water in a glass-stoppered container. Add 75 mL of hydrochloric acid and 5 mL of chloroform, and adjust to a faint iodine color (in the chloroform) by adding dilute potassium iodide or potassium iodate solution. If much iodine is liberated, use a stronger solution of potassium iodate than 0.01 *M* at first, making the final adjustment with the 0.01 *M* potassium iodate. Store in a dark place, and readjust to a faint iodine color as necessary.

Iodine and Potassium Iodide TS—Dissolve 500 mg of iodine and 1.5 g of potassium iodide in 25 mL of water.

Iodobromide TS—Dissolve 13.615 g of iodine, with the aid of heat, in 825 mL of glacial acetic acid that shows no reduction with dichromate and sulfuric acid. Cool, and titrate 25.0 mL of the solution with 0.1 *N* sodium thiosulfate VS, recording the volume consumed as *B*. Prepare another solution containing 3 mL of bromine in 200 mL of glacial acetic acid. To 5.0 mL of this solution add 10 mL of potassium iodide TS, and titrate with the 0.1 *N* sodium thiosulfate VS, recording the volume consumed as *C*. Calculate the quantity, *A*, of the bromine solution needed to double the halogen content of the remaining 800 mL of iodine solution by the formula:

$$800B/5C.$$

Add the calculated volume of bromine solution to the iodine solution, mix, and store in glass containers, protected from light.

Iodochloride TS—Dissolve 16.5 g of iodine monochloride in 1000 mL of glacial acetic acid.

Iodoplatinate TS—Dissolve 300 mg of platinum chloride in 97 mL of water. Immediately prior to use, add 3.5 mL of potassium iodide TS, and mix.

Iron-Phenol TS (Iron-Kober Reagent)—Dissolve 1.054 g of ferrous ammonium sulfate in 20 mL of water, and add 1 mL of sulfuric acid and 1 mL of 30 percent hydrogen peroxide. Mix, heat until effervescence ceases, and dilute with water to 50 mL. To 3 volumes of this solution contained in a volumetric flask add sulfuric acid, with cooling, to make 100 volumes. Purify phenol by distillation, discarding the first 10% and the last 5%, collecting the distillate, with exclusion of moisture, in a dry, tared glass-stoppered flask of about twice the volume of the phenol. Solidify the phenol in an ice bath, breaking the top crust with a glass rod to ensure complete crystallization. Weigh the flask and its contents, add to the phenol 1.13 times its weight of the iron-sulfuric acid solution prepared as directed, insert the stopper in the flask, and allow to stand, without cooling but with occasional mixing, until the phenol is liquefied. Shake the mixture vigorously until mixed, allow to stand in the dark for 16 to 24 hours, and again weigh the flask and its contents. To the mixture add 23.5% of its weight of a solution of 100 volumes of sulfuric acid in 110 volumes of water, mix, transfer to dry glass-stoppered bottles, and store in the dark, protected from atmospheric moisture. Use within 6 months. Dispense the reagent from a small-bore buret, arranged to exclude moisture, capable of delivering 1 mL in 30 seconds or less, and having no lubricant, other than reagent, on its stopcock. Wipe the buret tip with tissue before each addition.

Iron Salicylate TS—Dissolve 500 mg of ferric ammonium sulfate in 250 mL of water containing 10 mL of diluted sulfuric acid, and add water to make 500 mL. To 100 mL of the resulting solution add 50 mL of a 1.15% solution of sodium salicylate, 20 mL of diluted acetic acid, and 80 mL of a 13.6% solution of

sodium acetate, then add water to make 500 mL. Store in a well-closed container. Protect from light. Use within two weeks.

Lead Acetate TS—Dissolve 9.5 g of clear, transparent crystals of lead acetate in recently boiled water to make 100 mL. Store in well-stoppered bottles.

Lead Acetate TS, Alcoholic—Dissolve 2 g of clear, transparent crystals of lead acetate in alcohol to make 100 mL. Store in tight containers.

Lead Subacetate TS—Triturate 14 g of lead monoxide to a smooth paste with 10 mL of water, and transfer the mixture to a bottle, using an additional 10 mL of water for rinsing. Dissolve 22 g of lead acetate in 70 mL of water, and add the solution to the lead oxide mixture. Shake it vigorously for 5 minutes, then set it aside, shaking it frequently, during 7 days. Finally filter, and add enough recently boiled water through the filter to make 100 mL.

Lead Subacetate TS, Diluted—Dilute 3.25 mL of lead subacetate TS with water, recently boiled and cooled, to make 100 mL. Store in small, well-filled, tight containers.

Litmus TS—Digest 25 g of powdered litmus with three successive, 100-mL portions of boiling alcohol, continuing each extraction for about 1 hour. Filter, wash with alcohol, and discard the alcohol filtrate. Macerate the residue with about 25 mL of cold water for 4 hours, filter, and discard the filtrate. Finally digest the residue with 125 mL of boiling water for 1 hour, cool, and filter.

Locke-Ringer's Solution—See *Locke-Ringer's TS*.

Locke-Ringer's TS (Locke-Ringer's Solution)—

Sodium Chloride	9.0 g
Potassium Chloride	0.42 g
Calcium Chloride	0.24 g
Magnesium Chloride	0.2 g
Sodium Bicarbonate	0.5 g
Dextrose	0.5 g
Water, recently distilled from a hard-glass flask, a sufficient quantity, to make	1000 mL

Prepare fresh each day. The constituents (except the dextrose and the sodium bicarbonate) may be made up in stock solutions and diluted as needed.

Magnesia Mixture TS—Dissolve 5.5 g of magnesium chloride and 7 g of ammonium chloride in 65 mL of water, add 35 mL of ammonia TS, set the mixture aside for a few days in a well-stoppered bottle, and filter. If the solution is not perfectly clear, filter it before using.

Magnesium Sulfate TS—Dissolve 12 g of crystals of magnesium sulfate, selected for freedom from efflorescence, in water to make 100 mL.

Malachite Green TS—Dissolve 1 g of malachite green oxalate in 100 mL of glacial acetic acid.

Mallory's Stain—Dissolve 500 mg of water-soluble aniline blue, 2 g of orange G, and 2 g of oxalic acid in 100 mL of water.

Mayer's Reagent—See *Mercuric-Potassium Iodide TS*.

Mercuric Acetate TS—Dissolve 6.0 g of mercuric acetate in glacial acetic acid to make 100 mL. Store in tight containers, protected from direct sunlight.

Mercuric-Ammonium Thiocyanate TS—Dissolve 30 g of ammonium thiocyanate and 27 g of mercuric chloride in water to make 1000 mL.

Mercuric Bromide TS, Alcoholic—Dissolve 5 g of mercuric bromide in 100 mL of alcohol, employing gentle heat to facilitate solution. Store in glass containers, protected from light.

Mercuric Chloride TS—Dissolve 6.5 g of mercuric chloride in water to make 100 mL.

Mercuric Iodide TS (Valser's Reagent)—Slowly add potassium iodide solution (1 in 10) to red mercuric iodide until almost all of the latter is dissolved, and filter off the excess. A solution containing 10 g of potassium iodide in 100 mL dissolves approximately 14 g of HgI_2 at 20°.

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